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Influence of Cow Dung Extract Complemented with Nutrient Solution on the Growth Performance of Lettuce (*Lactuca sativa* L)

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A R T I C L E I N F O

Research Article

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A B S T R A C T

Limited studies have focused on plant growth performance using organic-based solutions complemented with mineral elements in a hydroponic system. Thus, this study aimed to investigate the growth performance of lettuce (*Lactuca sativa* L.) as influenced by cow dung extract combined with a hydroponic nutrient solution. Treatments considered as four different levels of aerated cow dung extracts (C), *viz.,* C_1 = 50 g l^{-1}, C_2 =100 g l^{-1}, C_3 =150 g l^{-1} and C_4 =200 g l^{-1} and four strengths of standard nutrient solution (S), *viz,* S_1 = 30% of standard nutrient solution, S_2 = 40% of standard nutrient solution, S_3 = 50% of standard nutrient solution and S_4 = 60% of standard nutrient solution. The experiment was carried out using a deep flow technique in a semi-greenhouse. Various growth and physiological parameters were measured in this experiment. The obtained data were subjected to statistical analysis with 4 replicates by analysis of variance (ANOVA) using SPSS. In the case of growth parameters, the tallest plant (23.54 cm), maximum number of leaves per plant (17.01) broadest leaf (13.32 cm), and the highest fresh weight (112.05 g/plant) were recorded from C_3 while the lowest in C_1. For hydroponic nutrient solution, the tallest plant (23.13 cm), the maximum number of leaves per plant (16.66), the widest leaf breath (14.17 cm), and the highest fresh weight (116.0 g/plant) were recorded from S_4 while the lowest in S_1. On the other hand, physiological traits viz. leaf area, net assimilation ratio, and relative growth rate were statistically higher in C_3 and lowest in C_1. In the nutrient solution, all physiological parameters were highest in S_4 and the lowest in S_1. In the case of the interaction effect, the highest fresh weight and almost all the parameters were found best in C_3S_4 and the lowest in C_1S_1. Therefore, the analysis showed that in terms of growth promotion properties hydroponic nutrient solution along with cow dung extract had a substantial impact and C_3S_4 was the most preferable treatment combination. Based on these findings, in a hydroponic system, an inorganic nutrient solution combined with organic liquid fertilizer derived from cow dung extract (as an alternative nutrient source) requires further improvements to achieve optimal growth and yield.

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Introduction

Safely feeding the ever-increasing population has become a rising challenge in responding to global environmental problems, and natural resource degradation, including soil degradation and biodiversity loss. Most estimates also indicate that climate change is likely to reduce agriculture productivity and production stability which already have high levels of food insecurity. Agriculture must undergo a significant transformation to meet the related challenges. Substitution of traditional farming systems with cost-effective integration of different production units has emerged as a potential solution. In respect to food security, balanced nutrition, and income generation, hydroponics is getting increased attention worldwide. Hydroponics, a soilless cultivation system, has emerged as an innovative and sustainable alternative to traditional agriculture, offering numerous advantages (Sasireka Rajendran et al., 2024). As a high-potential cultivation technique for growing safe vegetables, hydroponic is becoming increasingly relevant throughout the world. Soilless culture systems in greenhouses can reduce fertilizer application and nutrient leaching compared to soil-based systems. However, the major constraints on the adoption of the technology in Bangladesh by ordinary farmers are a shortage of technical knowledge and impute, principally hydroponics nutrient solution. Moreover, consumers are demanding higher-quality and safer food and are highly interested in organic products (Oudaet al., 2008). Hence, much attention has been paid in recent years to managing different organic waste resources to improve organic fertilizers through biological processes on a low-input as well as eco-friendly basis (Suthar, 2007). Cow dung is a potential source of organic matter, and analysis of representative cow dung slurry samples made at the Bangladesh Agricultural Research Institute (BARI) and Dhaka University (DU) has shown that cow dung slurry contains a considerable amount of both macro and micronutrients besides appreciable quantities of organic matter (Islam, 2006), which can be used in different ways in vegetable crop production. Productivity of hydroponics farming systems can be achieved through developing a method for using cow dung extract which is important resource of supplement organic matter in Bangladesh as here high animal densities are producing large volumes of compostable materials. But using organic nutrient solutions in hydroponics remains complex (Ahmed et al., 2021) and the direct use of organic waste without proper processing can be detrimental to plant growth (Leggo, 2015). Therefore, organic fertilizer has been microbially pre-processed before incorporation into hydroponic solutions (Atkin and Nichols, 2004). Treated extracts may also be used indirectly as fertilizer additives in liquid form. Before using in a direct method, it needs some decomposition. It has been reported that cow dung sludge contains certain concentrations of plant nutrients, making it useful as a liquid fertilizing medium if used with care. Before being used indirectly as fertilizer additives in liquid form, they would need to be diluted to ensure minimum plant damage. This dilution decreases the nutrient concentration necessary for plants. However, the use of organic manures alone cannot fulfill the crop nutrient requirements Kondapa*et* et al. (2009). Bokhtiar*et* et al. (2008) reported that organic manures when applied with chemical fertilizers, gave better yields than individual ones. The commercial formulation of cow dung extract as a liquid fertilizer needs to be used along with a hydroponic nutrient solution to promote plant nutrient utilization. Moreover, the proportion of different nutrient elements affects the tissue composition of vegetable crops. Though bio-diverse vegetable and spice crops can be grown sustainably over the years through hydroponics, it has been found that simple hydroponic techniques such as floating culture are successful in growing leafy vegetables like lettuce. Lettuce (*Lectuca sativa* L.) is a leafy vegetable of considerable agricultural and economic interest, and it is reported to respond well to fertilizer application. For that, lettuce can be considered a testing crop to test the suitability of cow dung extract as a fertilizer additive in liquid form. By considering the above literature, the present study aimed to find out the appropriate dose cow dung extract along with hydroponic nutrient solution on the growth and yield of hydroponic lettuce.

Materials and Methods

Experimental Site and Structure

Two years of repeated experiments were conducted from September 2019 to March 2020 and September 2020 to March 2021. It was conducted in the semi-greenhouse at the Horticulture Farm, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. The experiments were conducted in a structure using polyvinyl chloride (PVC) pipes. The structure consisted of four 5- feet lengths of 5-inch PVC pipe and a stand trellis made up of strong and durable steel. The stand measuring 3 feet x 3 feet x 2.5 feet where the four growing tubes were installed and each pipe has been considered as an experimental unit (Plate 1). Holes were made on the upper part of the pipe and the distance between two holes was 19.06 cm. Pipes had been placed horizontally on this stand, as the holding plants became more exposed to sunlight.

Experimental Design and Treatment

The two-factor experiment was conducted in a completely randomized design (CRD) with four replications. Factor A considered four different types of cow dung extract denoted as C, *viz.,* C_1 = Cow dung extract 50 g.L^{-1}, C_2= Cow dung extract 100 g.L^{-1}, C_3 Cow dung extract 150 g.L^{-1}, and C_4 = Cow dung extract 200 g.L^{-1} and factor B considered as four different strengths of standard nutrient solution [Rahman and Inden (2012)] used as a standard nutrient solution]denoted as S, *viz.,* S_1 = 30% strength of standard solution, S_2 = 40% strength of standard solution, S_3 = 50% strength of standard solution, and S_4 = 60% strength of standard solution. Loose leaf type lettuce (*Lactuca sativa* cv. 'Green Wave') was used as a planting material and eight plants were considered as an experimental unit.

Nutrient Solution Treatments

Nutrient solution is the most important component of the hydroponic system and in this present study, the treatment nutrient solution was prepared by mixing modified hydroponic standard solution and cow dung extract. The nutrient solution was prepared with distilled water and chemical-grade reagents. The ratio of Rahman and Inden (2012) solution were NO_3-N, P, K, Ca, Mg, and S of 17.05, 7.86, 8.94, 9.95, 6.0 and 6.0 meq. L-1, respectively. The rates of micronutrients were Fe, B, Zn, Cu, Mo, and Mn of 3.0, 0.5, 0.1, 0.03, 0.025, and 1.0 mgL-1, respectively for both the nutrient solutions. The cow dung extract was formulated by merging of following two different methods which are (Charoenpakdee, 2014; Peiris et al., 2015) where cow dung was used as a raw material organic source of nutrients and Mazim organic fertilizer (Mazim Agro Industries Ltd.) as a source of microbial inoculum.

pH and Electrical Conductivity of Solution:

The pH and EC values for all nutrient media were determined before use. The EC of each nutrient solution was about 2.0 dS /m, and the pH was adjusted at 5.5 to 6.5 using citric acid for the organic nutrient solutions, but for the inorganic solution, the pH was adjusted by using nitric and phosphoric acids (3: 1 v/v).

Harvesting and Data Collection

At random, three loose-leaf lettuce plants from each treatment were harvested after 42 days of sowing. Substrates in the roots of plants from substrate cultivation treatments were gently washed off with tap water. Data on different growth components, e.g., plant height, leaf breadth, leaf length, per plant leaf number, fresh weight, and dry matter of the plant were assessed to study morphological traits among treatments. Different physiological parameters [Leaf area (LA), leaf area ratio

(LAR), leaf mass ratio (LMR), root weight ratio (RWR), relative growth rate (RGR), and net assimilation rate (NAR)] were determined in the experiments. The parameters were measured according to Rahman and Inden (2012), as described below.

$$LAR=\frac{LA}{PDW} \quad (1)$$

Where LAR = leaf area ratio, LA = Leaf area (cm^2), PDW = plant dry weight (g).

$$LMR=\frac{LDW}{PDW} \quad (2)$$

where LMR = leaf mass ratio, LDW = leaf dry weight (g)

$$RWR=\frac{RDW}{PDW} \quad (3)$$

where RWR = root weight ratio, RDW = root dry weight (g).

$$RGR=\frac{(PDW1-PDW0)}{(t1-t0)}\times PDW_0 \quad (4)$$

where t = time, subscripts 0 and 1 refer to the transplanting and final harvest (days), respectively.

$$NAR=\frac{RGR}{LAR} \quad (5)$$

Statistical Analysis

Data from the two trials were combined and analyzed by one-way analysis of variance (ANOVA) using SPSS version 26.0, and differences among the means were determined by Tukey's HSD test at $P \leq 0.05$.

Results and Discussion

Plant Height

There were significant differences in plant height at 7, 14, 21, 28 and 42 days after transplanting (DAT) in respect of different treatments of cow dung extract (Figure 1) and nutrient solution (Figure 2). Results showed that plant height increased with the advancement of plant maturity. The tallest plants were obtained for C_3 at all times, while C_1 produced the shortest plants. It is revealed that plant height increased with an increasing dose of cow dung extract until a certain dose. It was also noticed that there was a higher amount of nitrogen in cow dung extract. Nitrogen slowly released from cow dung extract might have encouraged more vegetative growth in the plant at a later stage of growth. Scientists have reported that different levels of organic manure significantly increased plant height (Yadav and Malik, 2005). In the case of the nutrient solution, the tallest plants were found in S_4 and the shortest were found in S_1 at 7, 14, 21, 28, 35, and 42 DAT. These might be due to balanced nutrition during the growth period of lettuce and the readily available nitrogen for growth and development, resulting in the tallest plant. Similar results were observed by Tittonell et al. (2003) and Boroujerdnia and Ansari (2007).

Meanwhile, for the combination of cow dung extract and nutrient solution, plant height of lettuce significantly varied at 7, 14, 21, 28, 35 and 42 DAT (Table 1). At 7 DAT, the tallest plant (7.57 cm) was found in C_4S_4 and the shortest (5.15 cm) was found in C_1S_1. At 14 DAT, the shortest plant height observed was in C_1S_1 (6.78 cm) and tallest for C_3S_4 (12.45 cm) which is statistically similar to that of C_3S_3 (12.37 cm). But at 21, 28, 35 and 42 DAT the tallest plant was found in C_3S_4. Data presented in Table 1 indicated that cow dung extract and nutrient solution alone and their interaction significantly influenced plant height.

Number of Leaves

Significant variation was recorded for the number of leaves/plant of lettuce at 7, 14, 21, 28 35, and 42 days after transplanting (DAT) with the application of different levels of cow dung extract (Figure 3) and nutrient solution (Figure 4). The maximum number of leaves was found in C_3 and the minimum number of leaves per plant was in C_1. The results revealed that with an increase in the amount of cow dung extract until a certain dose, the number of leaves also increased. The increase in the number of leaves per plant might be due to the availability of nutrients in cow dung extract applied to the plant during the growth period of the crop and its role in enhancing the physical properties of the growing environment. Similarly, a higher number of leaves per plant was also obtained with cattle manure (Michael et al., 2012).

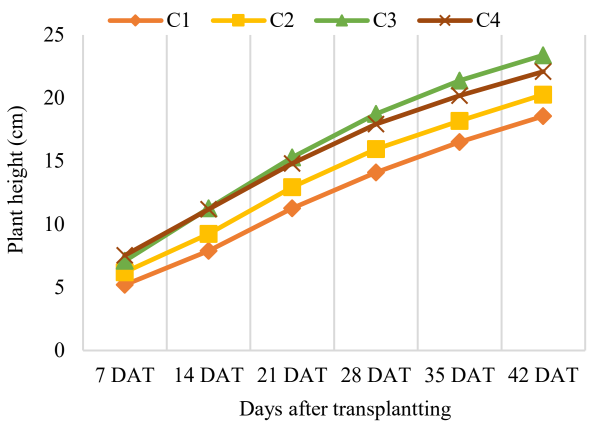

Figure 1. Main effects of cow dung extract on plant height at different days after transplanting (DAT)

Here, C_1 = 50 g dry cow dung equivalent extract per liter, C_2= 100 g dry cow dung equivalent extract per liter, C_3 = 150 g dry cow dung equivalent extract per liter, and C_4 = 200 g dry cow dung equivalent extract per liter. days after transplanting (DAT)

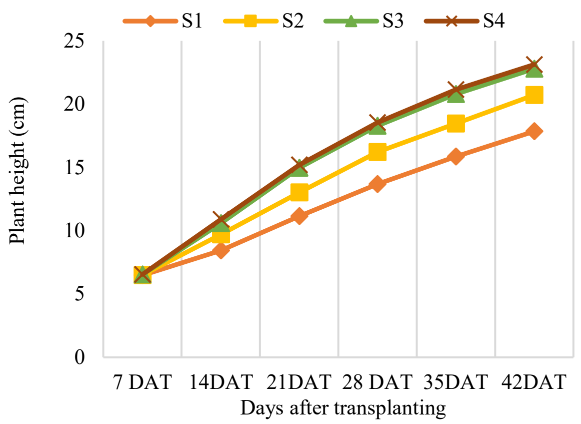

Figure 2. Main effects of nutrient solution on plant height at different days after transplanting (DAT)

Here, S_1 = 30%of the standard solution, S_2 = 40%of the standard solution, S_3 = 50%of the standard solution andS_4 = 60%of the standard solution. Days after transplanting (DAT)

Table 1. Combined effect of cow dung extract and nutrient solution on plant height of lettuce on different days after transplanting

Treatment	Plant height at different days after transplanting (DAT) (cm)					
	7DAT	14DAT	21DAT	28DAT	35DAT	42DAT
C_1S_1	5.15 d[z]	6.78 l	9.50 l	11.75 p	14.26 o	16.38 n
C_1S_2	5.17 d	7.60 k	11.04 k	14.03 n	16.04 m	18.03 l
C_1S_3	5.23 d	8.42 i	12.03 j	15.03 k	17.52 j	19.58 i
C_1S_4	5.24 d	8.83 h	12.52 i	15.47 i	18.25 i	20.27 g
C_2S_1	6.16 c	8.17 j	11.07 k	13.77 o	15.87 n	17.77 m
C_2S_2	6.18 c	9.18 g	12.51 i	15.27 j	17.53 j	20.03 h
C_2S_3	6.25 c	9.57 f	13.77 h	17.06 h	19.27 h	21.20 f
C_2S_4	6.26 c	10.03 e	14.35 f	17.68 f	20.05 f	22.15 e
C_3S_1	7.08 b	9.27 g	12.01 j	14.67 l	16.77 k	18.77 j
C_3S_2	7.08 b	11.01 d	14.51 e	18.02 e	20.48 e	22.77 d
C_3S_3	7.13 b	12.37 ab	17.27 b	21.02 b	24.02 b	26.27 a
C_3S_4	7.14 b	12.45 a	17.4 a	21.27 a	24.26a	26.37 a
C_4S_1	7.52 a	9.52 f	12.02 j	14.48 m	16.50 l	18.46 k
C_4S_2	7.54 a	11.01 d	14.02 g	17.52 g	19.79 g	22.03 e
C_4S_3	7.54 a	12.02 c	16.77 c	20.02 c	22.37 c	24.17 b
C_4S_4	7.57 a	12.26 b	16.51 d	19.75 d	22.03 d	23.74c
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[z]Means with different letter is significantly different by Tukey's test at $P \leq 0.05$. C_1 = 50 g dry cow dung equivalent extract per liter, C_2= 100 g dry cow dung equivalent extract per liter, C_3 = 150 g dry cow dung equivalent extract per liter, and C_4 = 200 g dry cow dung equivalent extract per liter. S_1 = 30%of the standard solution, S_2 = 40%of the standard solution, S_3 = 50%of the standard solution and S_4 = 60%of the standard solution. *P* represents the level of significance of one-way ANOVA.

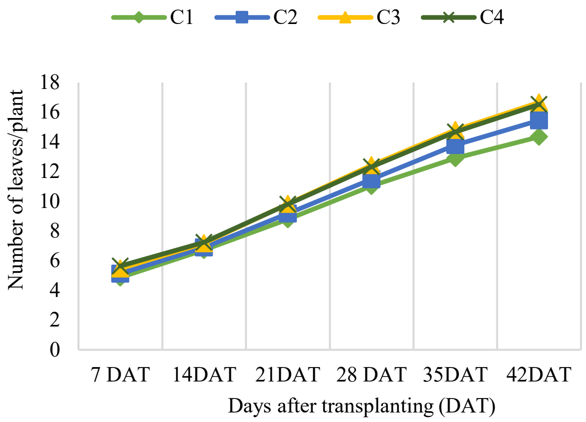

Figure 3. Main effects of cow dung extract on the number of leaves of lettuce on different days after transplanting

Here, C1 = 50 g dry cow dung equivalent extract per liter, C2= 100 g dry cow dung equivalent extract per liter, C3 = 150 g dry cow dung equivalent extract per liter, and C4 = 200 g dry cow dung equivalent extract per liter days after transplanting (DAT)

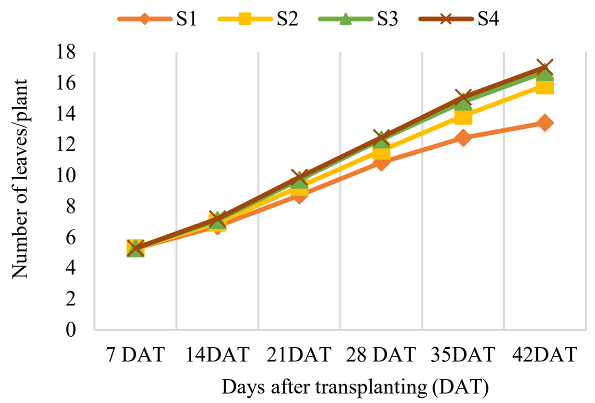

Figure 4. Main effects of cow dung extract and nutrient solution on no. of the leaf of lettuce on different days after transplanting

Here, S1 = 30%of the standard solution, S2 = 40%of the standard solution, S3 = 50%of the standard solution and S4 = 60%of the standard solution. Days after transplanting (DAT)

In the case of nutrient solution, the minimum number of leaves per plant at all dates was found in the plants grown in S_1 and the maximum number of leaves per plant was found in S_4 in all the cases. The reason might be the same as what was discussed in the case of plant height.

At 7 DAT, the maximum number of leaves/plant (5.65) was found in C_4S_4 and the minimum number of leaves/plant (4.85) was found in C_1S_1. At 14 DAT, the minimum number of leaves per plant observed in C_1S_1 (6.36) and the maximum number of leaves per plant for C_3S_4 (7.42) and C_4S_4 (7.42) were statistically similar. But at 21, 28, 35, and 42 DAT it was found that the maximum number of leaves/plant number of leaf in C_3S_4. The pH and EC with other properties were more favorable and ensured an appropriate condition for the elongation of the lettuce plant with optimum vegetative growth and the ultimate result was the maximum number of leaves per plant in C_3S_4.

Leaf Breath

The leaf breath of lettuce was significantly influenced by different treatments of cow dung (Figure 5). The results showed that the narrowest leaf breath at all dates was observed in the plants grown in C_1 and the widest leaf breath at all dates was found in plants grown in C_4. Optimum vegetative growth occurred due to the enhanced amount of nitrogen fertilizer that led to the growth of lettuce, and the ultimate result was the widest leaf. The findings previously obtained by Boroujerdnia and Ansari (2007) were comparable to the present analysis. Leaf breath differed significantly among the four treatments of nutrient solution at different DAT (Figure 6). The results revealed that the maximum leaf breath at all dates was found in plants grown in S_4 and the lowest leaf breath at all dates was observed in the plants grown in S_1.

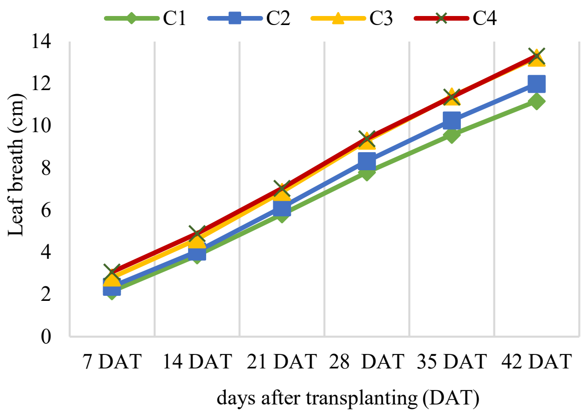

Figure 5. Main effects of cow dung extract on leaf breathe of lettuce on different days after transplanting

Here, C1 = 50 g dry cow dung equivalent extract per liter, C2= 100 g dry cow dung equivalent extract per liter, C3 = 150 g dry cow dung equivalent extract per liter, and C4 = 200 g dry cow dung equivalent extract per liter. days after transplanting (DAT)

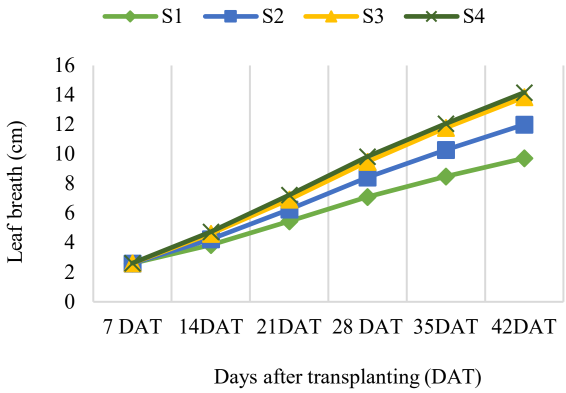

Figure 6. Main effects of nutrient solution on leaf breathe of lettuce at different days after transplanting

Here, S1 = 30%of the standard solution, S2 = 40%of the standard solution, S3 = 50%of the standard solution and S4 = 60%of the standard solution. Days after transplanting (DAT)

Table 2. Combined effect of cow dung extract and nutrient solution on leaf number of lettuce on different days after transplanting

Treatment	Number of leaves on different days after transplanting (DAT) (cm)					
	7DAT	14DAT	21DAT	28DAT	35DAT	42DAT
C_1S_1	4.85 d[z]	6.36 i	8.08 i	10.05 n	11.29 n	12.13 n
C_1S_2	4.85 d	6.65 g	8.67 h	10.90 l	12.78 l	14.44 j
C_1S_3	4.88 d	6.89 f	9.03 g	11.43 i	13.52 i	15.03 i
C_1S_4	4.88 d	7.01 e	9.38 e	11.76 h	14.03 g	15.76 g
C_2S_1	5.13 c	6.58 h	8.62 h	10.76 m	12.07 m	13.04 m
C_2S_2	5.13 c	6.84 f	9.05 g	11.39 ij	13.77 h	15.57 h
C_2S_3	5.13 c	7.01 e	9.43 e	11.84 g	14.25 f	16.30 f
C_2S_4	5.14 c	7.17 c	9.58 d	11.97 f	15.02 d	16.88 d
C_3S_1	5.46 b	6.88 f	9.03 g	11.25 k	13.29 j	14.35 k
C_3S_2	5.47 b	7.09 d	9.64 C	12.18 e	14.49 e	16.67 e
C_3S_3	5.48 b	7.28 b	10.24 b	13.09 b	15.70 ab	17.77 ab
C_3S_4	5.48 b	7.42 a	10.36 a	13.21 a	15.76 a	17.83 a
C_4S_1	5.63 a	7.02 e	9.13 f	11.33 j	13.07 k	14.12 l
C_4S_2	5.64 a	7.17 c	9.69 c	12.12 e	14.45 e	16.70 e
C_4S_3	5.65 a	7.32 b	10.19 b	12.99 c	15.66 b	17.70 b
C_4S_4	5.65 a	7.42 a	10.23 b	12.86 d	15.53 c	17.57 e
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[z]Means with different letter is significantly different by Tukey's test at $P \leq 0.05$. C_1 = 50 g dry cow dung equivalent extract per liter, C_2= 100 g dry cow dung equivalent extract per liter, C_3 = 150 g dry cow dung equivalent extract per liter, and C_4 = 200 g dry cow dung equivalent extract per liter. S_1 = 30% of the standard solution, S_2 = 40% of the standard solution, S_3 = 50% of the standard solution and S_4 = 60% of the standard solution. *P* represents the level of significance of one-way ANOVA

In the case of the combined effect of cow dung extract and nutrient solution, a significant variation was found at 7, 14, 21, 28, 35, and 42 DAT (Table 3) in terms of leaf breath of lettuce. At 7 DAT, the broadest leaf (3.08 cm) was found in C_4S_4 and the leaf breath (2.16 cm) was found in C_1S_1. At 14 DAT, the narrowest leaf breath was observed in C_1S_1 (3.21 cm) and the widest in C_4S_3 (5.13 cm) and C_4S_4 (5.10 cm), which were statistically similar. But at 21 and 28 DAT it was found that the maximum leaf breath was in C_3S_4 and C_4S_4. At 35 and 42 DAT it was found that the widest leaf breath was in C_3S_4 and C_3S_3. The widest leaf breath, almost all dates, was found in C_3S_4 and the lowest was found in C_1S_1.

Fresh Weight of Lettuce

Marketable lettuce quality is determined primarily by the size of the plant and its fresh weight. There was an insignificant difference in fresh weight at transplanting time but differed at harvesting among the treatments (Table 4). At harvest time, for cow dung extract total fresh weight was found to be higher in C_3 (116.0g / plant) the lowest fresh weight was found in C_1 (85.19 g / plant), and for the nutrient solution it was found to be higher in S_4 (112.05 g / plant) the lowest fresh weight found in S_1 (80.91 g / plant). This might be due to a higher number of leaves and leaf breath by an appropriate amount of nutrients and suitable conditions. Higher fresh weight of leaf was found (71.02 g/plant) in C_3 and the lowest was in C_1 (53.63 g/plant) in response to cow dung extract and for the nutrient solution the highest and lowest weight was found in S_4 (75.73 g/plant) and S_1 (46.99 g/plant) respectively. It was revealed that balanced nutrition and optimum nitrogen level ensured maximum vegetative growth resulting highest fresh weight/ plant. The results obtained earlier by Tittonell et al. (2003), were similar to the present study. In the case of root, higher fresh weight (33.18 g/plant) was found in C_4 which is statistically similar to that of C_3 and minimum fresh weight (23.41 g/plant) was in C_1 for cow dung extract. In response to the nutrient solution higher root fresh weight was found in S_4 (29.92 g/plant) and lowest in S_1 (26.47 g/plant).

Table 3. Combined effect of cow dung extract and nutrient solution on leaf breath of lettuce at different days after transplanting

Treatment	Leaf breath at different days after transplanting (DAT) (cm)					
	7DAT	14DAT	21DAT	28DAT	35DAT	42DAT
C_1S_1	2.16 d[z]	3.21 k	4.54 l	5.88 k	6.99 m	7.93 m
C_1S_2	2.17 d	3.60 j	5.18 j	7.14 i	8.75 k	10.17 k
C_1S_3	2.16 d	4.21 g	6.45 g	8.80 f	10.92 g	12.86 g
C_1S_4	2.17 d	4.44 e	6.92 d	9.39 d	11.66 e	13.75 d
C_2S_1	2.38 c	3.61 j	5.14 k	6.71 j	8.06 l	9.22 l
C_2S_2	2.38 c	3.94 i	5.89 i	7.99 g	9.78 h	11.38 h
C_2S_3	2.38 c	4.21 g	6.61 f	9.11 e	11.36 f	13.42 e
C_2S_4	2.39 c	4.36 f	6.94 d	9.54 c	11.84 d	13.95 c
C_3S_1	2.80 b	4.15 h	5.90 i	7.77 h	9.34 j	10.72 j
C_3S_2	2.81 b	4.54 d	6.76 e	9.18 e	11.29 f	13.22 f
C_3S_3	2.82 b	4.81 c	7.29 b	10.00 b	12.44 ab	14.65 a
C_3S_4	2.82 b	5.01 b	7.54 a	10.23 a	12.52 a	14.68 a
C_4S_1	3.05 a	4.49 de	6.23 h	8.05 g	9.56 i	11.01 i
C_4S_2	3.06 a	4.84 c	7.06 c	9.38 d	11.34 f	1324 f
C_4S_3	3.07 a	5.13 a	7.37 b	10.05 b	12.34 bc	14.45 b
C_4S_4	3.08 a	5.10 a	7.51 a	10.12 ab	12.27 c	14.29 b
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[z]Means with different letter is significantly different by Tukey's test at $P \leq 0.05$. C_1 = 50 g dry cow dung equivalent extract per liter, C_2= 100 g dry cow dung equivalent extract per liter, C_3 = 150 g dry cow dung equivalent extract per liter, and C_4 = 200 g dry cow dung equivalent extract per liter. S_1 = 30% of the standard solution, S_2 = 40% of the standard solution, S_3 = 50% of the standard solution and S_4 = 60% of the standard solution. P represents the level of significance of one-way ANOVA

Table 4. Main effects of cow dung extract and nutrient solution on fresh weight of lettuce

Treatment	Fresh weight (FW) per plant at harvesting time (g)			
	Total	Leaf	Stem	Root
Cow dung extract (CS)				
C_1	85.19 d[z]	53.63 d	8.16 d	23.41 d
C_2	95.26 c	58.66 c	9.12 c	27.46 b
C_3	116.0 a	71.02 a	11.84 a	29.98 a
C_4	110.0 b	69.30 b	10.74 b	33.18 a
Nutrient solution (S)				
S_1	80.91 d	46.99 d	7.45 d	26.47 d
S_2	96.95 c	59.74 c	8.93 c	28.28 c
S_3	111.00 b	70.16 b	11 48 b	29.36 b
S_4	112.05 a	75.73 a	11.98 a	29.92 a
Level of significance (P)				
C	<0.001	0.002	<0.001	<0.001
S	<0.001	<0.001	<0.001	0.146

[z]Means with different letter is significantly different by Tukey's test at $P \leq 0.05$. C_1 = 50 g dry cow dung equivalent extract per liter, C_2= 100 g dry cow dung equivalent extract per liter, C_3 = 150 g dry cow dung equivalent extract per liter, and C_4 = 200 g dry cow dung equivalent extract per liter. S1 = 30% of the standard solution, S_2 = 40% of the standard solution, S_3 = 50% of the standard solution and S_4 = 60% of the standard solution. *P* represents the level of significance of one-way ANOVA

In the case of the combined effect of cow dung extract and nutrient solution, a significant variation in fresh weight was found at harvesting (Table 5). The highest fresh weight in all cases was found in C_3S_3 which was statistically similar to that of C_4S_4 and the lowest was found in C_1S_1.

Lettuce quality is determined primarily by the size of the plant and its fresh weight. Insignificant difference in fresh weight at transplanting time but differed at harvesting among the treatments (Table 4). At harvest time, for cow dung extract total fresh weight was found higher in C_3 (116.0g / plant) the lowest fresh weight was found in C_1 (85.19 g / plant), and for nutrient solution, it was found to be higher in S_4 (112.05 g / plant) the lowest fresh weight found in S_1 (80.91 g / plant). This might be due to higher number of leaf and leaf breath by an appropriate amount of nutrients and suitable conditions. Higher fresh weight of leaf was found (71.02 g/plant) in C_3 and the lowest was in C_1 (53.63 g/plant) in response to cow dung extract and for the nutrient solution the highest and lowest weight was found in S_4 (75.73 g/plant) and S_1 (46.99 g/plant) respectively. It was revealed that balanced nutrition and optimum level of nitrogen ensured maximum vegetative growth resulting highest fresh weight/ plant. The results obtained earlier by Tittonell et al., (2003), were similar with the present study. In case of root, higher fresh weight (33.18 g/plant) found in C_4 which is statistically similar that of C_3 and minimum fresh weight (23.41 g/plant) was in C_1 for cow dung extract. In response to the nutrient solution higher root fresh weight was found in S_4 (29.92 g/plant) and lowest in S_1 (26.47 g/plant).

In the case of the combined effect of cow dung extract and nutrient solution, a significant variation in fresh weight was found at harvesting (Table 5). The highest fresh weight in all cases was found in C_3S_3 which was statistically similar to that of C_4S_4 and the lowest were found in C_1S_1.

Table 5. Combined effects of cow dung extract and nutrient solution on fresh weight of lettuce

Treatment	Fresh weight (FW) per plant at harvesting time (g)			
	Total	Leaf	Stem	Root
C_1S_1	63.26 j[z]	36.91 j	5.14 l	21.20 j
C_1S_2	78.75 i	49.63 h	6.83 k	22.28 ij
C_1S_3	91.44 gh	57.57 f	9.75 f	24.12 hi
C_1S_4	1.0731E e	70.39 d	10.89 e	26.03 fgh
C_2S_1	75.50 i	42.53 i	7.16 k	25.80 gh
C_2S_2	89.33 h	54.30 fg	7.97 j	27.05 fg
C_2S_3	100.83 f	62.88 e	10.02 f	27.91 ef
C_2S_4	115.37 c	74.93 c	11.34 d	29.10 de
C_3S_1	93.96 g	53.90 g	9.07 g	30.98 C
C_3S_2	111.57 C	67.89 d	10.89 de	32.78 bc
C_3S_3	130.59 a	81.91 a	13.55 a	35.13 a
C_3S_4	128.05 a	80.39 ab	13.83 a	33.83 ab
C_4S_1	90.94 gh	54.62 fg	8.42 h	27.90 ef
C_4S_2	108.15 de	67.11 d	10.03 f	31.01 C
C_4S_3	119.82 b	78.27 bc	12.59 b	30.30 d
C_4S_4	119.82 b	77.20 bc	11.90 c	30.72 d
P	<0.001	<0.001	<0.001	<0.001

[z]Means with different letter is significantly different by Tukey's test at $P \leq 0.05$. C_1 = 50 g dry cow dung equivalent extract per liter, C_2= 100 g dry cow dung equivalent extract per liter, C_3 = 150 g dry cow dung equivalent extract per liter, and C_4 = 200 g dry cow dung equivalent extract per liter. S1 = 30% of the standard solution, S_2 = 40% of the standard solution, S_3 = 50% of the standard solution and S_4 = 60% of the standard solution. *P* represents the level of significance of one-way ANOVA

Table 6. Main effects of cow dung extract and nutrient solution on dry weight of lettuce

Treatment	Dry weight (DW) per plant at harvesting time (g)			
	Total	Leaf	Stem	Root
Cow dung extract (CS)				
C_1	4.90 d[z]	2.99 d	0.51 d	1.40 d
C_2	5.43 c	3.21 c	0.58 c	1.64 c
C_3	6.24 b	3.95 a	0.74 a	1.75 b
C_4	6.68 a	3.82 b	0.68 b	1.98 a
Nutrient solution (S)				
S_1	4.64 d	2.61 d	0.47 d	1.60 d
S_2	5.54 c	3.28 c	0.56 c	1.69 c
S_3	6.37 b	3.90 b	0.72 b	1.74 b
S_4	6.73 a	4.20 a	0.75 a	1.77 a
Level of significance (P)				
C	<0.001	0.002	<0.001	<0.001
S	<0.001	<0.001	<0.001	0.148

[z]Means with different letters is significantly different by Tukey's test at $P \leq 0.05$. C_1 = 50 g dry cow dung equivalent extract per liter, C_2= 100 g dry cow dung equivalent extract per liter, C_3 = 150 g dry cow dung equivalent extract per liter, and C_4 = 200 g dry cow dung equivalent extract per liter. S1 = 30% of the standard solution, S_2 = 40% of the standard solution, S_3 = 50% of the standard solution and S_4 = 60% of the standard solution. *P* represents the level of significance of one-way ANOVA

Dry Weight of Lettuce

Plant dry weights of lettuce did not vary significantly at transplanting time but difference in harvesting across treatments of cow dung (Table 6). The average total dry weight was higher in C4 (6.68 g/plant) and the lowest dry weight was found in C1 (4.90 g/plant). In the case of dry leaf higher weight was found in C3 (3.95 g/plant) and the lowest leaf dry weight was found in C1 (2.99 g/plant). Dry weight of stem found greater (0.74 g/plant) in C3 and the lowest dry weight of stem (0.51 g/plant) found in C1., On the other hand, a higher root dry weight (1.98 g/plant) was found in C4, and a minimum dry weight (1.40 g/plant) was found in C1.

Lettuce's dry weight differed significantly from the four treatments of nutrient solution (Table 6) as well. Upon drying the harvested lettuce plant, the highest total dry weight was in S4 (6.73 g/plant), and the lowest was in S1 (4.64 g/plant). In this case, the dry weight of the leaf was found higher (4.20 g/plant) in S4, and the lowest leaf dry weight (2.61 g/plant) was found in S1. The dry weight of the stem was found greater (0.75 g/plant) in S4 and the lowest dry weight of the stem (0.47 g/plant) was found in S1. In the case of root, a higher dry weight (1.77 g/plant) was found in S4, and a minimum dry weight (1.60 g/plant) was found in S1. This might be because of the proportion of nutrient supply in the plants. Andriolo et al. (2005) stated that lettuce growth was affected by different strengths of nutrient solution. The results of this experiment are also compatible with that.

In the event of a combined interaction of cow dung extract and nutrient solution (Table 7) the lowest plant dry weight for all cases was found in C_1S_1 and the highest was found in C_3S_3 which was statistically similar to that of C_3S_4 for dry weight of leaf, stem, and total. Maximum vegetative growth has been helped to ensure the highest dry weight/plant and that can be obtained because of the interaction impact of various levels of inorganic and organic nutrition.

Table 7. Combined effects of cow dung extract and nutrient solution on dry weight of lettuce

Treatment	Dry weight (DW) per plant at harvesting time (g)			
	Total	Leaf	Stem	Root
C_1S_1	3.62 m^z	2.03 k	0.32 m	1.27 l
C_1S_2	4.52 k	2.76 i	0.43 l	1.33 k
C_1S_3	5.27 ih	3.22 g	0.61 g	1.44 j
C_1S_4	6.21 e	3.95 c	0.69 e	1.56 i
C_2S_1	4.32 l	2.32 j	0.45 k	1.54 i
C_2S_2	5.14 j	3.02 h	0.51 j	1.62 h
C_2S_3	5.76 g	3.46 f	0.63 f	1.67 g
C_2S_4	6.51 d	4.04 c	0.72 d	1.74 f
C_3S_1	5.37 h	3.01 h	0.55 h	1.79 e
C_3S_2	6.43 d	3.78 d	0.68 e	1.97 c
C_3S_3	7.43 a	4.55 a	0.86 a	2.10 a
C_3S_4	7.43 a	4.49 a	0.87 a	2.07 b
C_4S_1	5.26 i	3.05 h	0.53 i	1.68 g
C_4S_2	6.06 f	3.57 e	0.63 f	1.86 d
C_4S_3	6.91 b	4.36 b	0.79 b	1.75nf
C_4S_4	6.76 c	4.28 b	0.75 c	1.73 f
P	<0.001	<0.001	<0.001	<0.001

^{z}Means with different letter is significantly different by Tukey's test at $P \leq 0.05$. C_1 = 50 g dry cow dung equivalent extract per liter, C_2= 100 g dry cow dung equivalent extract per liter, C_3 = 150 g dry cow dung equivalent extract per liter, and C_4 = 200 g dry cow dung equivalent extract per liter. S1 = 30% of the standard solution, S_2 = 40% of the standard solution, S_3 = 50% of the standard solution and S_4 = 60% of the standard solution. *P* represents the level of significance of one-way ANOVA

Table 8. Main effects of cow dung extract and nutrient solution on physiological traits of lettuce

Treatment	LA (cm^2)	LMR ($g\ g^{-1}$)	LAR ($cm^2\ g^{-1}$)	RWR ($g\ g^{-1}$)	NAR ($g\ cm^{-2}\ d^{-1}$)	RGR ($g\ g^{-1}\ d^{-1}$)
Cow dung extract						
C_1	250.00 d^z	0.916 ab	157.404 b	0.08534 bc	0.0000028 c	0.00045 d
C_2	270.00 c	0.907 c	161.20 a	0.092878 a	0.0000029 c	0.00047 c
C_3	310.33 b	0.921 a	120.9 c	0.079325 c	0.0000061 b	0.00073 b
C_4	321.33 a	0.913 bc	106.79 d	0.087490 ab	0.0000080 a	0.00085 a
Solution (S)						
S_1	268.25 d	0.917 a	133.51 b	0.08382 a	0.00000478 b	0.00060 c
S_2	278.67 c	0.914 ab	133.83 b	0.08530 a	0.00000499 ab	0.00062 d
S_3	297.25 b	0.913 ab	138.32 a	0.08650 a	0.00000506 a	0.00064 a
S_4	307.5 a	0.910 b	140.31 a	0.08941 a	0.00000507 a	0.00066 a
Level of significance (p)						
C	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
S	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

^{z}Means with different letters is significantly different by Tukey's test at $P \leq 0.05$. LA = Leaf area; LMR = Leaf mass ratio; LAR = Leaf area ratio; RWR = Root weight ratio; NAR = Net assimilation rate; RGR = Relative growth rate. C_1 = 50 g dry cow dung equivalent extract per liter, C_2= 100 g dry cow dung equivalent extract per liter, C_3 = 150 g dry cow dung equivalent extract per liter, and C_4 = 200 g dry cow dung equivalent extract per liter. S_1 = 30% of the standard solution, S_2 = 40% of the standard solution, S_3 = 50% of the standard solution and S_4 = 60% of the standard solution. *P* represents the level of significance of one-way ANOVA.

Physiological Growth Traits

Significant variation of physiological growth parameters of lettuce plants was recorded with application of different levels of cow dung extract and nutrient solution (Table 8). In case of leaf area (LA), the higher (321.33 cm2) leaf area was found in the plants grown in C_4 and the lower (250 cm2) was found in C_1. Leaf area is an important determinant of light interception and consequently of transpiration, photosynthesis and plant productivity (Dufour, L. and Guérin, V. (2005). In case of Leaf Mass Ratio (LMR), the higher (0.921 g g-1) Leaf Mass Ratio (LMR) was found in C_3 and the lower (0.907 g g-1) was found in C_2. Higher LMR is one of the important criteria for producing higher metabolites. Prieto et al. (2007) reported that increased LMR gave the plants an increased ability to intercept light. In case of Leaf Area Ratio (LAR), the lower (106.79 cm2 g-1) Leaf Area Ratio (LAR) was found in C4 while the highest (161.20 cm2 g-1) was found in C_2. Lower LAR is one of the important criteria for producing higher metabolites. In case of Root Weight Ratio (RWR), the lower RWR (0.0793250 g g-1) was found in C_3 while the higher (0.092878 g g-1) was found in C_2. Lower RWR is one of the important criteria for producing higher metabolites. Net assimilation rate (NAR) and relative growth rate of lettuce of lettuce was also significantly affected by cow dung extract (Table 8). The highest net assimilation of lettuce was found in C_4 (0.0000080 gcm-2d-1). On the other hand, C_1 (0.0000028 gcm-2d1) showed the lowest net assimilation rate. It might be due to this experiment's environmental conditions, especially high luminosity and temperature, Prieto et al. (2007) reported that increased NAR gave the plants an increased ability to intercept light.

Table 9. Combined effects of cow dung extract and nutrient solution on physiological traits of lettuce

Treatment	LA (cm^2)	LAR ($cm^2\ g^{-1}$)	LMR ($g\ g^{-1}$)	RWR ($g\ g^{-1}$)	NAR ($g\ cm^{-2}\ d^{-1}$)	RGR ($g\ g^{-1}\ d^{-1}$)
C_1S_1	231.0 i	152.800 c	0.925 a	0.079207 ab	0.00000281 d	0.000426 h
C_1S_2	239.0 hi	152.241 c	0.917 abc	0.082885 ab	0.00000 292 d	0.000446 gh
C_1S_3	261.0 fg	161.114 abc	0.913 abc	0.08656 ab	0.00000 285 d	0.000460 fgh
C_1S_4	269.0 ef	163.461 ab	0.907 bc	0.092732 a	0.00000 286 d	0.000466 fg
C_2S_1	250.0 hg	154.255 bc	0.908 abc	0.091955 ab	0.00000 298 d	0.000460 fgh
C_2S_2	262.0 f	158.679 abc	0.906 abc	0.091433 ab	0.00000295 d	0.000470 fg
C_2S_3	278.0 e	164.688 a	0.906 c	0.093572 a	0.00000291 d	0.000479 fg
C_2S_4	278.0 d	167.202 a	0.905 c	0.094552 a	0.00000294 d	0.000493 f
C_3S_1	290.0 d	121.936 d	0.92 ab	0.075247 b	0.00000555 c	0.000676 e
C_3S_2	301.33 C	119.664 d	0.921 abc	0.078997 ab	0.00000559 bc	0.000716 d
C_3S_3	319.0 b	119.942 d	0.921 abc	0.078917 ab	0.00000631 b	0.000756 c
C_3S_4	319.0 a	120.826 d	0.916 abc	0.084138 ab	0.00000646 b	0.000780c
C_4S_1	301.33 c	105.053 e	0.911 abc	0.088908 ab	0.00000779 a	0.000816 b
C_4S_2	312.33 bc	104.745 e	0.912 abc	0.087865 ab	0.00000810 a	0.000850 ab
C_4S_3	331.0 a	107.566 e	0.913 abc	0.086971 ab	0.00000815 a	0.000880 a
C_4S_4	340.0 a	109.784 e	0.914 abc	0.086216 ab	0.00000803 a	0.000880 a
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[z]Means with different letter is significantly different by Tukey's test at $P \leq 0.05$. LA = Leaf area; LMR = Leaf mass ratio; LAR = Leaf area ratio; RWR = Root weight ratio; NAR = Net assimilation rate; RGR = Relative growth rate. C_1 = 50 g dry cow dung equivalent extract per liter, C_2= 100 g dry cow dung equivalent extract per liter, C_3 = 150 g dry cow dung equivalent extract per liter, and C_4 = 200 g dry cow dung equivalent extract per liter. S_1 = 30% of the standard solution, S_2 = 40% of the standard solution, S_3 = 50% of the standard solution and S_4 = 60% of the standard solution. *P* represents the level of significance of one-way ANOVA.

Plate 1. Growing lettuce plants on a hydroponic structure


Plate 2. Harvesting of lettuce for data collection

The highest relative growth rate (RGR) of lettuce was found in C_4 (0.00085 g g-1d-1). On the other hand, C_1 (0.00045 g g-1d-1) showed the lowest relative growth rate. The results revealed that the highest relative growth rate was found in S_2. Meanwhile S1 denoted the lowest relative growth rate.

The physiological growth parameters of lettuce had been substantially affected by the combination of cow dung extract and nutrient solution treatment (Table 9). (Ahmed *et al.,* 2021), reported organic fertilizers and factors related to the mineral composition and the availability of the nutrients in the solution that influence leaf number and area. For the leaf area (LA), the highest leaf area (LA) was found in C_4S_4 and the lowest was found in C_1S_1.

The highest leaf area ratio (LAR) of lettuce was found in C_2S_4 and the lowest was found in C_4S_2. In the case of leaf mass ratio, the lowest leaf mass ratio was found in C_2S_4 while the higher was found in C_1S_1. The lowest root weight ratio of lettuce was found in C_3S_1. On the other hand, the highest root weight ratio was found in C_2S_4. Lettuce's net assimilation rate and relative growth rate are the lowest in C_1S_1 and are 0.00000281 g cm^{-2} d^{-1} and 0.000426 g $g^{-1}d^{-1}$ respectively. On the other hand, the maximum net assimilation rate was demonstrated in C_4S_3 (0.00000815 g cm^{-2} d^{-1}) and the relative growth rate in C_4S_4 (0.000880 g $g^{-1}d^{-1}$).

Conclusions

The results showed that the compositional amounts of the various levels of cow dung extract and nutrient solution had a substantial impact on the vegetative growth and physiological growth of lettuce. The combination of C_3 (150 g dry cow dung equivalent extract per liter) and S_4 (60 percent of the standard solution) would be most favorable for the growth performance of leaf lettuce. This allows the conclusion that even though organic production is sustainable and organic products have more interest in terms of human health and the environment, using cow dung extract as an organic nutrient solution in hydroponics remains complex. To assess the efficacy and to overcome the limitations of such systems, more research is needed. Additionally, a careful choice of organic fertilizers is recommended, to optimize organic nutrient solutions for hydroponic lettuce production to achieve better growth and yield.

Declarations

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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